PART I - ADMINISTRATIVE

Section 1. General administrative information

Title of project
Induction of Precocious Sexual Maturity and Enhanced Egg Production in Fish

BPA project number: 20046
Contract renewal date (mm/yyyy): ☐ **Multiple actions?**

Business name of agency, institution or organization requesting funding
University of Idaho

Business acronym (if appropriate) UI

Proposal contact person or principal investigator:

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NPPC Program Measure Number(s) which this project addresses
7.2, 7.4D (1994 Columbia River Basin Fish and Wildlife Program)

FWS/NMFS Biological Opinion Number(s) which this project addresses

Other planning document references

Short description
Determine if female fish sexual maturity can be achieved earlier (thus increased reproductive capacity and reduced generation time) due to a drastic growth rate increase (3.5 times normal) induced by the use of somatotropin.

Target species
Salmonid, White Sturgeon

Section 2. Sorting and evaluation

Subbasin
Systemwide

Evaluation Process Sort

CBFWA caucus	Special evaluation process	ISRP project type
Mark one or more caucus	If your project fits either of these processes, mark one or both	Mark one or more categories
☒ Anadromous fish	☒ Multi-year (milestone-based	☐ Watershed councils/model watersheds

☒ Resident fish ☐ Wildlife	evaluation) ☐ Watershed project evaluation	☐ Information dissemination ☐ Operation & maintenance ☐ New construction ☒ Research & monitoring ☐ Implementation & management ☐ Wildlife habitat acquisitions

Section 3. Relationships to other Bonneville projects

Umbrella / sub-proposal relationships. List umbrella project first.

Project #	Project title/description

Other dependent or critically-related projects

Project #	Project title/description	Nature of relationship
1	Intracytoplasmic Sperm Injection: Genetic Retrieval from Single Sperm	Project Participant
2	Endocrine Control of Ovarian Development in Salmonids	Project Participant
3	Analyzing Genetic and Behavioral Changes During Salmonid Domestication	Project Participant
5	Enhancement of Salmonid Gamete Quality by Manipulation of Intracellular ATP	Project Participant
6	Viral Vaccines and Effects on Reproductive Status	Project Participant

Section 4. Objectives, tasks and schedules

Past accomplishments

Year	Accomplishment	Met biological objectives?
None	None	None

Objectives and tasks

Obj 1,2,3	Objective	Task a,b,c	Task
1	Test the effects of various dosage levels of bovine somatotropin on physiological growth in young white sturgeon.	a	Conduct growth trials with 40,80 and 120 mcg somatotropin/g body weight to determine optimal growth conditions.
2	Test the effects of various dosage levels of bovine somatotropin on physiological growth and its relationship to a) the timing of sexual maturity, b) fecundity	b	Conduct trout trials to determine optimal growth conditions and carry out the sexual maturity, fecundity and egg quality studies.

	and c) egg quality in rainbow trout.		
3	Investigate the relationship of growth hormone, IGF-I and IGF-II to growth rate and sexual maturity in rainbow trout and white sturgeon.	c	Conduct trout trials to determine optimal growth conditions and carry out the sexual maturity, fecundity and egg quality studies.
4	Test the effects of various dosage levels of bovine somatotropin on physiological growth and its relationship to a) the timing of sexual maturity, b) fecundity and c) egg quality in white sturgeon.	d	Conduct the necessary animal trials with graded levels of somatotropin, other than that optimal for growth to determine the effect of these levels on sexual maturity fecundity and egg quality.
5	Test the effects of various dosage levels of bovine somatotropin on the periodicity of white sturgeon spawning.	e	Carry out the trials to determine the periodicity of spawning.

Objective schedules and costs

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	11/1999	2/2000	Optimal growth conditions		9.00%
2	11/1999	4/2001	Determination of earlier sexual maturity in salmonids if successful	Yes	25.00%
3	6/2000	2/2002	An understanding of the growth stimulating hormones and onset of sexual maturity		19.00%
4	1/2002	10/2003	Determination of earlier sexual maturity in sturgeon if successful	Yes	28.00%
5	1/2002	9/2004	Determination of periodicity of sturgeon spawning	Yes	19.00%
				Total	**100.00**

Schedule constraints

Completion date
FY 2004

Section 5. Budget

FY99 project budget (BPA obligated):

FY2000 budget by line item

Item	Note	% of total	FY2000
Personnel	Post-Doctoral Research Assoc.		33,000
Fringe benefits	Post-Doctoral Research Assoc.		9,405
Supplies, materials, non-expendable property			34,450
Operations & maintenance			
Capital acquisitions or			

improvements (e.g. land, buildings, major equip.)			
NEPA costs			
Construction-related support			
PIT tags	# of tags: 200		580
Travel	One meeting. Travel to off-campus fish laboratory		3,900
Indirect costs	31.5%		47,145
Subcontractor			
Other	Technical Res. Assoc. (100%) Fringe, Tech. Res. Assoc. (34.5%) Aquaculture Core Hormone Assay Core Administrative Core		28,500 9,832 15,000 10,000 5,000
TOTAL BPA FY2000 BUDGET REQUEST			196,812

Cost sharing

Organization	Item or service provided	% total project cost (incl. BPA)	Amount ($)
None	None	None	None
Total project cost (including BPA portion)			

Outyear costs

	FY2001	FY02	FY03	FY04
Total budget	$191,400	$189,000	$179,500	$162,500

Section 6. References

Watershed?	Reference
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PART II - NARRATIVE

Section 7. Abstract

The overall objective is to develop a bovine somatotropin (bST) treatment regime that will result in the increased reproductive capacity and reduced generation time of female salmonid (rainbow trout) and white sturgeon. We hypothesize that increased growth rate is highly correlated with earlier sexual maturity, as is the case with mammals, and have already demonstrated that the growth of white sturgeon can be increased to 3.5 fold with bST. The general nature of the work will be to induce an accelerated growth rate (of several magnitudes) through the use of sustained-release bST and determine if sexual maturity can be achieved much earlier. Our goal is to develop this application for enhanced fish production through increased reproductive capacity and reduced generation time, as well as elucidate the hormonal relationships underlying the association between growth and sexual maturity in fish.

We will work with two different species of Pacific Northwest fish that are representative of diverse physiological traits. White sturgeon will be studied due to their slow growth and delayed timing of sexual maturity, and rainbow trout will serve as a representative of the faster-growing cold water salmonids. Sexual maturity will be determined, egg quantity and quality measurements will be made, and related hormone levels will be studied. This work can be achieved in a reasonable time with rainbow trout, and an assay model will be developed utilizing juvenile, nearly mature, and sexually mature female sturgeon in order to facilitate the timely generation of data necessary for this slower growing species.

Section 8. Project description

a. Technical and/or scientific background

The role of reproduction, more specifically female reproductive capacity (maximal egg production per female per unit of time), is critical to the maintenance and reestablishment of endangered species of wild fish, as well as the efficient propagation of other wild and cultured fish. Increased reproductive capacity in female fish can be achieved by shortening the time to sexual maturity and by increasing egg production per spawning.

The growth time required to achieve sexual maturity is a major problem with certain fish, such as white sturgeon (*Acipenser transmontanus*), which may take 11-20 years to achieve their first spawn [Conte et al., 1988] and, thereafter, may spawn at a frequency of every 2-8 years. Decreasing the time to the first spawn (sexual maturity) and increasing the frequency of spawning would be of considerable benefit to the management of many wild fish. [Bromage et al., 1992].

Rainbow trout are highly fecund, in contrast to sturgeon, producing a single batch of 2000-3000 4-5 mm eggs per kilogram of body weight each year. Oocyte development in trout comprises two phases, primary growth and secondary growth, each of which can be divided into a number of stages [Tyler et al., 1994]. Growth occurs principally in the secondary growth phase, during vitellogenesis, when oocytes increase in size from less than 0.5 mm in diameter up to 4-5 mm [Tyler, 1991, Tyler et al., 1996]. Most of this growth is accounted for by the uptake of the extra-ovarian protein vitellogenin from the blood. Fecundity is normally determined by early vitellogenesis, and subsequently there is little, if any change in the number of oocytes recruited in to the secondary growth phase through to ovulation nine months later [Tyler, 1990]. Little is known in most animals about the factors affecting recruitment or about the mechanisms controlling oocyte growth and development. Even less is known about the determinants of fecundity. Therefore investigation of the interaction between growth and reproduction and the potential role of growth hormone is being emphasized.

Growth hormone (GH) is a principal regulator of somatic growth in salmonids. The observation of some increased growth rate has been indicated in coho salmon and rainbow trout by investigators using

somatotropin from bovine [McLean et al., 1990], porcine [McLean et al., 1992], ovine [Foster et al., 1991] and fish [Agellon et al., 1988] sources. Our laboratory has demonstrated that the mode of administration and dosage pattern of growth hormone is critical in determining the magnitude of growth response of rainbow trout [Garber, et al., 1995], and that growth rates can readily be at least doubled with certain treatments [Schelling et al., 1997}. Our more recent work with 350 gram white sturgeon has indicated a tremendous increase in growth rate of over 3.5-fold by the administration of exogenous bovine growth hormone [Schelling, et al., submitted], and we have observed similar results in channel catfish. Evidence for a direct relationship between female growth rate, or size, and sexual maturity in cattle and other mammals is well established [Murphy, et al., 1991; Gong et al., 1991], and it is suggested to exist in teleost fishes by observations of 1) elevated plasma GH levels in sexually mature fish, 2) increased plasma GH levels following administration of sex steroid hormones and 3) the stimulatory effect of GH on the production of gonadal steroid hormones [Bromage et al., 1992; Bromage and Cumaranatunga, 1988; Bromage et al., 1990a; Sumpter et al., 1991; Le Gac et al., 1993; Moriyama et al., 1997; Bjornsson et al., 1994; Bjornsson, 1997; Holloway and Leatherland, 1997].

On the basis of mammalian work and some fish work, it appears that the growth stimulating effect of GH is integrated with that of (IGF-I). GH stimulates protein synthesis and improves feed conversion during growth. The hormone also promotes lipid and glycogen breakdown as well as gluconeogenesis, functions which are probably of great importance during fasting when GH levels are seen to increase. It is becoming clear that GH is an important and multi-functional hormone in salmonids, and probably most other fish, and a central mediator of seasonal changes in physiology and behavior. Its role in osmoregulation and feeding behavior is being elucidated [Sakamoto et al., 1993; McCormick, 1995]. A key question remaining is whether GH is involved in the regulation of sexual maturation in fish.

Correlation of GH levels to gonadal development has been studied in Atlantic salmon [Bjornsson et al., 1994], chum salmon [Kakisawa et al., 1995] and rainbow trout [Sumpter et al., 1991; Holloway and Leatherland, 1997]. GH receptors have been identified in rainbow trout gonads [Yao et al., 1991; Le Gac et al., 1992]. GH modulates steroid production by rainbow trout gonads *in vitro* [Sing et al., 1988; Le Gac et al., 1992], and raises estradiol and testosterone levels in juvenile rainbow trout [Van Der Kraak et al., 1990]. Endogenous GH levels are elevated during sexual maturation in rainbow trout [Sumpter et al., 1991; Foucher et al., 1992; Le Gac et al., 1992] as well as in Atlantic salmon [Bjornsson et al., 1994] and chum salmon [Kakisawa et al., 1995]. As salmonids naturally stop feeding during sexual maturation, the increased GH levels have been interpreted to be a response to fasting [Sumpter et al, 1991; Foucher et al., 1992]. Gonadal steroid treatment of fed and fasted rainbow trout indicate that GH levels are correlated to sexual maturation, timing of ovulation and steroid levels [Bjornsson et al., 1994; Holloway and Leatherland, 1997]. Gonadal steroid priming of immature rainbow trout increases pituitary responsiveness *in vitro* to GH secretagogues such as gonadotropin releasing hormone (GnRH) [Holloway and Leatherland 1997a] and the excitatory amino acid N-methyl-DL-aspartate [Flett et al., 1994; Holloway and Leatherland 1997a,c]. Similar enhanced responsiveness has been found in sexually maturing rainbow trout [Holloway and Leatherland 1997a], offering a possible explanation for increased GH levels during sexual maturation.

Administration of growth hormone to teleost fishes results in elevated levels of IGF-I mRNA [Cao et al., 1989; Duan, et al., 1994] and plasma IGF-I [Moriyama 1994, 1995]. Little work has been done with IGF-II in fish. The relationship of plasma IGF-I to sexual maturity has been investigated in precociously maturing salmon [Moriyama et al., 1997], while hepatic IGF-I mRNA levels have been compared in juvenile vs. mature trout [Shamblott and Chen, 1993]. In coho salmon, IGF-I mRNA expression has been observed in the gonad [Duan, 1995] and IGF-I appears to be effective on ovarian steroidogenesis [Maestro et al., 1995]. IGF-I induces maturation of oocytes in red seabream by its action as a potent inducer of germinal vesicle breakdown (GVBD) [Kagawa, 1994]. IGF-I expression in catfish tissues has been studied by McRory and Sherwood [1994], but no other investigations have been conducted in catfish to observe for a relationship between growth and sexual maturity. No investigations, to date, have examined the presence or role of IGF-I and IGF-II in sturgeon. Further study is warranted to examine the physiological significance of GH and IGF-I and IGF-II to sexual maturity. The use of exogenous bST over-rides the negative feedback effects of IGF-I on GH release from the pituitary in rainbow trout [Perez-Sanchez et al., 1992; Blaise et al., 1995].

White sturgeon typically reach sexual maturity between the ages of 11-20 years. It is size, however, rather than age, which appears to be important to the onset of sexual maturity since captive female sturgeon have been observed to reach earlier sexual maturity through control of nutrition, water temperature and photo period [personal communication with Serge Doroshov]. The reproductive physiology and development of sturgeon exhibit many features common to amphibians and mammals, such as the primary structure of the gonadotropin-releasing hormone [Lescheid et al., 1995], the nucleotide and derived amino acid sequences in the vitellogenin gene [Bidwell and Carlson, 1995], and embryonic development [Detlaff et al., 1993]. Comparison between the conformations of sturgeon somatotropin and somatotropins isolated from several mammalian species, including bovine and human, indicates a close relationship between these molecules [Bewley and Papkoff, 1987].

b. Rationale and significance to Regional Programs

The role of reproduction, more specifically female reproductive capacity (maximal egg production per female per unit of time), is critical to the maintenance and reestablishment of endangered species and reduced numbers of wild fish. Increased reproductive capacity in female fish can be achieved by shortening the time to sexual maturity and by increasing egg production per spawning.

The growth time required to achieve sexual maturity is a major problem with certain fish, such as sturgeon, which may take 11-20 years to achieve their fist spawn. Furthermore, sexually mature female sturgeon have a spawning frequency of 2-8 years. This has contributed to the decline and endangerment of fish populations such as the white sturgeon, *Acipenser transmontanus*, population in the Kootenai River ecosystem in Idaho, Montana and British Columbia which was listed as endangered on September 6, 1994 (59 FR 45989) under the authority of the Endangered Species Act of 1973. This action was taken in response to the virtual absence of natural recruitment during the past two decades, and an aging and declining population size [Anders and Richards, 1996]. Research efforts aimed at decreasing the time to the first spawn (sexual maturity) and increasing the frequency of spawning would be of considerable benefit to the management of many wild fish as well as the efficiency of commercially produced fish.

The detailed physiological relationship between growth rate and time of sexual maturity and egg production per spawn is not well understood in fish. Our laboratory has demonstrated that the mode of administration and dosage pattern of growth hormone is critical in determining the magnitude of growth response of rainbow trout, and that growth rates can readily be at least doubled with certain treatments. Our more recent work with 350 gram white sturgeon has indicated a tremendous increase in growth rate of over 3.5-fold by the administration of bovine growth hormone (bST), and we have observed similar results in channel catfish. The use of bST is partially based on its current availability due to its commercial production and use in the cattle industry. Another advantage is that this material can be readily administered in a sustained-release form. The impact of this rather drastic accelerated growth rate will be investigated with primary focus on age and size of fish at sexual maturity, and the quantity and quality of egg production of female salmonid (rainbow trout) and white sturgeon.

The direct relationship between female growth rate, or size, and sexual maturity in cattle and most other mammals is well established and is suggested to also exist in fish. **The hypothesis for this work is that the increased growth rate of bovine somatotropin treated female fish will have a significant impact upon a) the minimal time required for the first spawn and b) the number and quality of eggs produced at first and subsequent spawns.**

c. Relationships to other projects

The current status of research investigating the relationship between physiological growth and onset of sexual maturity in fish is in an advanced infancy stage. Growth hormone appears to be a principal regulator of somatic growth in salmonids and its growth stimulating effects are probably integrated with that of IGF-I. Increased levels of GH and IGF-I have been reported to correlate with sexual maturation in trout and salmon, but no studies have been conducted on the kinetics of exogenous GH administration on IGF-I and IGF-II release into the bloodstream.

Recent studies reporting the production of IGF-I at the level of the ovary suggest a possible mechanism for growth-induced sexual maturation. Further study is certainly warranted to examine the physiological significance of GH and IGF-I and IGF-II to sexual maturity.

Most of the studies on the relationship between growth and sexual maturity are being conducted in salmonids while minimal information exists for sturgeon. Researchers at the University of California (personal communication) have observed that by controlling several environmental cues and by maximizing nutritional growth, female sturgeon can reach their first spawn earlier than the 11-20 years typical for wild fish. This certainly suggests that attempts to regulate sexual maturity with growth stimulants may greatly impact the reproductive capacity of sturgeon and play a critical role in the maintenance and reestablishment of endangered species of wild fish.

d. Project history (for ongoing projects)

(Replace this text with your response in paragraph form)

e. Proposal objectives

To test the hypothesis that the increased growth rate of bovine somatotropin treated female fish will have a significant impact upon (a) the minimal time required for the first spawn and (b) the number and quality of eggs produced at first spawn and subsequent spawns, we will work with two different species that are representative of various physiological traits and/or fish use traits. White sturgeon will be studied due to their slow growth and delayed sexual maturity and rainbow trout will be a representative of the fast-growing cold water salmonids. The general nature of the study will be to induce an accelerated growth rate through the use of bST and follow the fish through sexual maturity to collect eggs for quantitative and qualitative measurements. Furthermore, to provide insight into the mechanism(s) by which endocrines of the pituitary-gonadal axis effect sexual maturity, circulating and gonadal levels of IGF-I and IGF-II will be correlated with the egg quality and quantity data.

Objective 1: Test the effects of various dosage levels of bovine somatotropin on physiological growth in young white sturgeon.

Objective 2: Test the effects of various dosage levels of bovine somatotropin on physiological growth and its relationship to a) the timing of sexual maturity, b) fecundity and c) egg quality in rainbow trout.

Objective 3: Investigate the relationship of growth hormone, IGF-I and IGF-II to growth rate and sexual maturity in rainbow trout and white sturgeon.

Objective 4: Test the effects of various dosage levels of bovine somatotropin on physiological growth and its relationship to a) the timing of sexual maturity, b) fecundity and c) egg quality in white sturgeon.

Objective 5: Test the effects of various dosage levels of bovine somatotropin on the periodicity of white sturgeon spawning.

f. Methods

Somatotropin effect on growth in juvenile sturgeon. Our laboratory has demonstrated dramatically increased growth rates in rainbow trout, channel catfish and white sturgeon using sustained release bST. However, the optimal dosage conditions have not been studied in sturgeon. Our first objective will involve intraperitoneal injections of various bST dosages (0, 40, 80,120,160 Φg/g body weight) of 150 juvenile sturgeon at 3-week intervals for at least one year to measure growth rates (weight and length). The fish will be maintained in an outdoor concrete run supplied with continuous water flow of 15L/min, a constant temperature of 15EC, and natural photo period. The information gained will be used to understand

physiological growth in sturgeon and will facilitate knowledge for which range of bST dosage will be needed in our study of more mature sturgeon.

Determining stage of ovarian development in sturgeon

This parameter provides direct information on the ovarian development and allows us to examine female stock recruitment into vitellogenesis and final ovarian maturation. Ovarian biopsies will be collected from fish anesthetized in tanks containing 50-100 mg/L of MS-222. Each fish will be placed on a hooded stretcher ventral side up, with a flow of fresh water maintained across the gills. A 2-3 cm incision will be made slightly lateral to the ventral mid-line and small (approximately 2 cm^3) piece of gonadal tissue will be removed and preserved in 10% buffered formalin. Histological processing of the ovarian biopsies will include dehydration in a series of alcohols, clearing in xylenes, embedding in paraffin, sectioning at a thickness of 6 microns, and staining slides by periodic acid Schiff reagent differentiating glycoprotein structures (egg chorion and yolk platelets). Microscopic examination and analyses of slides will be based on previous studies of the ovarian histology in wild white sturgeon females [Chapman et al., 1987; Doroshov et al., 1991]. The stage of ovarian development will be determined by a score based upon ten distinct parameters, characterizing advanced development and differentiation of the ovarian follicle.

Score 0: Gonads contain clusters of gonial cells, connective and adipose tissues. During the biopsy the ovary is small and the gonadal tissue is a narrow ovigerous "ribbon."

Score 1: Oocytes enter cytoplasmic growth phase, and a thin PAS-positive basal lamina forms around each egg, separated from gonial clusters (which are still present). The cytoplasm is basophillic and the nucleus contains a few darkly stained nucleoli. The gross anatomy of the gonad is similar to score 0.

Score 2: Cytoplasm of the oocytes contains non-staining cortical vesicles. The basal lamina is thick and separates theca from granulosa layers. The granulosa layer contains only a few undifferentiated cells. Cytoplasm remains basophilic and there are still some patches of gonial cells in the ovarian tissue. The gonad is still similar to earlier stages although ovigerous folds are obvious and extend throughout the lateral side of the ovary.

Score 3: There are two, distinctly different in size, populations of oocytes: one is similar to the above described oocytes, and another is enlarged and possesses a less basophilic cytoplasm and fewer vesicles in the cytoplasm cortex. The follicular epithelium (granulosa) in the larger sized oocytes begins mitotic proliferation, and the outer follicular layer (theca) has some vascularization. The network of blood capillaries transports yolk precursor, vitellogenin, and oxygen to the developing eggs. A perinuclear framework of rough endoplasmic reticulum and oil droplets is formed. During this stage the oocytes become visible by the unaided eye, and appear to be translucent spheres dispersed throughout the ovigeous folds.

Score 4: The distinguishing feature of this score is the differentiation of the first, PAS-positive, layer of the egg chorion, zona radiata 1. Granulosa cells increase in thickness and become more cuboidal. The well differentiated thecal layer is vascularized. The oocytes have a very light basophilic (sometimes slightly eosinophilic) cytoplasm. At this stage, the developing oocytes are visible as very small white spheres embedded in the ovarian folds.

Score 5: In addition to the differentiated granulosa layer and a one layered chorion, there are small yolk platelets in the cytoplasm, recognized by their oval shape and eosinophilic or PAS-positive staining. The eggs in the ovary are seen as slightly larger spheres, while to yellowish in color.

Score 6 and 7: Differentiation of the second and third PAS-positive layers of chorion, correspond respectively to scores of 6 and 7. As egg size increases, the density and size of yolk platelets also increase. The ovary greatly enlarges due to intense oocyte growth. The individual egg size is about 1 mm.

Score 8: This score is given to females that have vitellogenic oocytes that are starting to darken in color as melanin pigment is deposited in the egg cortex. When this process begins, the oocytes are approximately 1.5 mm in diameter.

Score 9: During this stage, the nucleus (GV) moves off-center towards the animal pole. The animal pole contains small, round, yolk platelets, while the vegetal hemisphere of the egg contains larger, oval-shaped, yolk platelets and numerous medium-sized oil droplets. Small PAS-positive cortical vesicles (true cortical alveoli) appear as a single layer in the cytoplasm, adjacent to the oocyte membrane. During the time between score 8 and 9, the eggs darken and grow another 1 mm. The ovary is now full of large black eggs, about 3 mm in size.

Score 10: The structure of the oocyte is similar to score 9, but the nucleus has now migrated into the egg cortex near the animal pole and is almost completely submersed in the small, round, yolk platelets.

The scoring system described above clearly discriminates all stages of vitellogenesis. In defining the development of sexual maturity, we will consider all females with a score of 4 to have initiated the formation of the egg chorion (zona radiata 1) but with no yolk deposition apparent. Females with a score ranging from 5 to 8 are vitellogenic. Broodstock with a score of 9 or 10 are approaching final ovarian maturity.

Induction of spawning in sturgeon. In captivity, the reproductive development in a large number of maturing female sturgeon remains arrested at the previtellogenic stage. Even in those females that advance to spawning, oocyte maturation and ovulation does not occur spontaneously, necessitating the hormonal induction of spawning events [Moberg and Doroshov, 1996]. Optimal conditions for the timing of induced spawning include control of important environmental cues, namely, seasonal photo period and water temperature [Moberg and Doroshov, 1996]. Using the method outlined by Van Eenennaam et al. (1996), females will be injected (IM) with a priming dose of ovulation-inducing hormone (LHRHa at 10% of the total dose) and a resolving dose 12 hours later. Ovulation is expected 20-24 hours after the second injection at a holding temperature of 15-16EC. Females are anesthetized in 100 ppm MS-222 for 15 minutes, and their eggs are removed by aseptic caesarian surgery (10-12 cm incision). Anesthesia is monitored during surgery lasting 40-60 minutes by alternating water supply to the gills between fresh water and 50 ppm MS-222 solution. The incision is closed by internal and external stitches (PDS suture, Ethicon). Fish are injected with antibiotics (oxytetracycline, 5 mg/kg). Primary healing of the incision occurs within 1-2 months and complete healing in 4-5 months.

Data collection from spawned females will include: estimation of the percent of eggs released into the body cavity at caesarian section (% ovulation); latency time (hours after the second injection to the time when eggs were first observed in the spawning tank); total number of eggs collected determined volumetrically, egg diameter measured by image analysis.

Fecundity

Actual fecundity is defined as the number of ripe or mature eggs produced by female fish which can be artificially stripped from the fish. The term relative-fecundity is used to express number of eggs produced for each unit of post-stripped fish weight. Mean egg diameter and total volume of water-hardened eggs will used to measure egg productivity. Under commercial conditions, the eggs of rainbow trout are ovulated but not oviposited; they remain in the body cavity until they are artificially stripped from the fish. During the period of retention in the body cavity, the eggs undergo a ripening process. To optimized the chance for high rates of fertilization, eggs will be stripped between 4-10 days after ovulation (Springate et al., 1984).

Egg Quality Measurements

Egg quality will be assessed by measuring egg volume since this provides the most detailed comparisons of eggs from fish on different treatments [Bromage, et al., 1992]. Egg diameter will be determined by measuring to the nearest 0.05 mm by use of a stereoscopic binocular microscope fitted with an eyepiece graticule. Volumes will be estimated according to the formula for the volume of a sphere ($4/3\pi r^3$). Good quality eggs are defined as those which exhibit low levels of mortality at fertilization, eyeing, hatch and first-feeding and those which produce the fastest-growing and healthiest fry and older fish.

Measurement of IGF-I and IGF-II in plasma

Plasma IGF-I and IGF-II levels will be determined using modifications of a radioimmunoassay (RIA) procedure developed by Moryiama et al. (1994). Briefly, in the case of IGF-I, it will be separated from IGF binding proteins by acidification and separation using size-exclusion HPLC. RIA will be performed using recombinant salmon IGF-I (rsIGF-I) as tracer and standard. We have conducted the iodination and RIA procedures in several species, numerous times in our laboratory, therefore, we will optimize the conditions using proven techniques. Iodination of rsIGF-I will be conducted using chloramine-T and RIA procedure utilizes a double-antibody procedure. The IGF-II determination will be an analogous procedure. The rsIGFs, as well as the primary antibodies, will be acquired from GroPep Ltd..

Measurement of IGF-I and IGF-II in the ovary

Immunocytochemical methods for determining IGF-I in ovaries have been developed by Kawaga et al. (1995) using a polyclonal antibody against rsIGF-I. Briefly, ovarian tissue will be removed from aneshetized fish, cut into small pieces, fixed in Bouin's solution, dehydtrated, and embedded in paraffin. Serial 10 Φm sections will be cut, deparaffinized, and processed for immunocytochemical localization of IGF-I. Immunocytochemical staining will be performed using a streptavidin-biotin peroxidase kit.. Sections will first be incubated with 3% hydrogen peroxidase for 10 min to remove endogenous peroxidase staining. Sections will then be washed with phosphate-buffered saline (PBS) and exposed to 10% normal goat serum for 10 min to diminish nonspecific staining. Next, sections will be incubated with anti-rsIGF-I at a dilution of 1:1000 for 30 min, washed 3X with PBS, then incubated for 10 min with the second antibody (biotinylated anti-rabbit IgG) and again washed 3X with PBS. The sections will then be incubated with streptavidin-linked horseradish peroxidase for 10 min. After a final wash with PBS, peroxidase avtivity will be revealed with a solution of 0.01% hydrogen peroxidase-0.05% 3, 3'-diaminobenzidine tetrahydrochloride. An analogous procedure will be developed for IGF-II. Microscopic examination will determine the comparison between maturational stages of the oocytes and cellular location of IGF-I and IGF-II production.

g. Facilities and equipment

Animal facilities

A substantial variety of fish rearing facilities are available for this project on campus and at the UI Fish Culture Experiment Station in Hagerman, Idaho. These facilities range from smaller aquariums for short-term rearing, to mid-size tanks, to large tanks, to raceways for long-term rearing of coldwater fish. The aquariums are closed systems and all of the other tanks and raceways have an abundant supply of 15° C high quality spring water at the Hagerman facility.

The animal facilities are well equipped for the feeding, rearing, handling, pit tagging and surgical procedures proposed for this work.

Laboratories

Well equipped chemical, physiology and radioisotope laboratories are all available to carry out all of the proposed procedures. Purchase of major equipment and instrumentation is not required.

Animal sources

High quality, healthy rainbow trout and white sturgeon are readily available through a cooperative arrangement from the College of Southern Idaho Fisheries Program. These fish are available in a wide variety of sizes.

Collaborators and cooperators

In addition to the College of Southern Idaho cooperation as a source of fish, arrangements are being made with commercial aquaculture fisheries for field studies or large growing facilities as the need arises. Collaborations are in place with Monsanto Company as the source of high quality, sustained-release form of bovine growth hormone.

h. Budget

The work will require a full-time Post-Doctoral Research Associate and Technical Research Associate to work as a major part of the team to conduct the growth and physiology work and properly carry out the laboratory work. Materials and supplies for the animal work and laboratory work will be somewhat higher the first year than subsequent years. There will be a limited amount of non-expendable property purchased the first year. This will be reduced considerably the second year and be none thereafter. Only 200 PIT tags will be needed the first year and about half that many in subsequent years. Travel expenses include one scientific meeting and frequent trips to the off-campus fish laboratory in Hagerman Idaho. The budget also includes aquaculture core, hormone assay core and administrative core items. These funds support the aquaculture center operations for animal handling, the hormone laboratory for the required hormone assay specialized equipment, and general administrative funding for the management of the umbrella program. Indirect costs are based on the standard percentage established by the College of Agriculture of the University of Idaho.

Section 9. Key personnel

The principle investigator and co-investigator have extensive research experience between them in the fields of growth and development, somatotropin research and fish growth. Dr. Gerald Schelling is a Professor in the Department of Animal and Veterinary Science at the University of Idaho. He has conducted considerable research on the effects of somatotropin on the growth, body composition and metabolism of mammals, and more recently with fish. His laboratory has demonstrated the drastic growth response of fish to exogenous somatotropin. Dr. Ronald Hardy is Director of the University of Idaho Fish Culture Experiment Station. He has conducted extensive research on the nutrition and growth of trout and salmon. The two researchers have ongoing collaborative projects with trout and sturgeon.

Principal Investigator
Dr. Gerald T. Schelling, Professor, 0.15 FTE,

Ph. D., Animal Nutrition and Growth, University of Illinois, 1968.
M.S., Animal Nutrition, University of Illinois, 1964.
B.S., Animal Science, University of Illinois, 1963.

Professor, Dept. Anim. And Vet. Sci., University of Idaho.
Professor and Head, Dept. Anim. Sci., University of Idaho.
Assoc. Professor and Professor, Dept. Anim. Sci., Texas A&M University.
Asst. Professor and Assoc. Professor, Dept. Anim. Sci., University of Kentucky.

Schelling, G.T., R.A. Roeder, E.L. Brannon and J.C. Byatt. 1997. Stimulated growth in rainbow trout administered bovine somatotropin. J. Anim. Sci. (Suppl. 1) 75:172 .

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Co-Investigator
Dr. Ronald W. Hardy, Professor, 0.10 FTE

Ph. D., Fisheries. University of Washington, 1978.
M.S., Nutrition. Washington State University, 1973.
B.S., Zoology. University of Washington, 1969.

Professor, University of Idaho, Dept. Animal & Vet. Science
Affiliate Professor, University of Washington, School of Fisheries
Supervisory Research Chemist, Northwest Fisheries Center, NMFS, Seattle, WA.
Research Assistant Professor, University of Washington, School of Fisheries.

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Hardy, R. W. 1995. Current Issues in Salmonid Nutrition. Pp. 26-35 *In* Nutrition and Utilization Technology in Aquaculture, C. E. Lim and D. J. Sessa, eds. AOCS Press, Champaign, Illinois.

Post-Doctoral Research Associate
To be hired. 1.0 FTE
A Ph.D. degree and training in the area of fundamental fish reproductive physiology and endocrinology are required. This individual will be responsible for the daily operations in the overall conduction of the research. They will also be responsible for conducting and/or training the Technical Research Associate to conduct the highly technical aspects of the research. They will also be responsible for evaluating data and preparing reports on the progress of the research.

Technical Research Associate
To be hired. 1.0 FTE
A M.S. degree and training in the area of animal physiology and/or biochemistry are required. This individual will be responsible for conducting routine assays and techniques, and data handling in support of the research.

Section 10. Information/technology transfer

The information and technology resulting from this work will first be reported at scientific meetings and working group meetings in order to provide rapid information transfer. Subsequent publication in scientific and applied journals will proceed in a timely fashion.

Congratulations!